Neuron, Vol. 29, 567–570, March, 2001, Copyright ©2001 by Cell Press

The Grass Roots of Synapse Suppression

Minireview

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Not counting recreational use, the effects of marijuana on the brain have been under active investigation for at least the past 30 years. Important milestones in the elucidation of the physiological actions of psychoactive substances from *cannabis sativa* included the discovery of neuronal receptor proteins for cannabinoids and the existence of endogenous cannabinoid substances (Sullivan, 2000). In this issue of *Neuron*, two papers appear (Kreitzer and Regehr, 2001; Ohno-Shosaku et al., 2001), which along with another in *Nature* (Wilson and Nicoll, 2001), provide new and important insight into the detailed mechanisms by which endogenous cannabinoids exert their effects on nervous physiology. These papers report that endogenous cannabinoids released from postsynaptic neurons after depolarization act on presynaptic terminals to suppress subsequent neurotransmitter release, driving the synapse into an altered state.

The broad behavioral effects of cannabinoid intoxication such as impaired learning and memory, depressed cognitive skills, decreased motor coordination, and alterations in pain perception and emotional state, likely reflect its widespread action in many brain regions. Indeed, cannabinoid receptors are highly expressed throughout the central nervous system (Sullivan, 2000). CB1, a G protein–linked cannabinoid receptor, is expressed on the presynaptic terminals of inhibitory interneurons in the hippocampus and on presynaptic parallel and climbing fibers throughout the molecular layer in the cerebellum. To date, two endogenous cannabinoids have been isolated, the phospholipid derivatives anandamide and *sn*-2 arachidonylglycerol (2-AG) (Devane et al., 1992; Stella et al., 1997). While there is no doubt that many of their physiological roles still await discovery, endogenous cannabinoids are known to have significant effects on synaptic physiology. Specifically, 2-AG depresses release of the transmitters glutamate, γ-amino butyric acid (GABA), and acetylcholine in the hippocampus (Sullivan, 2000). Many downstream effects likely result from such an action, one of which is that a decrease in glutamate release decreases the effectiveness of stimuli in the induction of long-term potentiation and long-term depression (Lévénès et al., 1998; Misner and Sullivan, 1999), two forms of synaptic plasticity thought to be involved in learning and memory.

Some GABAergic inhibitory synapses display an interesting property known as *d*epolarization-induced suppression of *i*nhibition, or DSI, during which their function is suppressed following depolarization of the postsynaptic cell (Llano et al., 1991; Alger and Pitler, 1995). The actual trigger for DSI appears to be a rise in postsynaptic calcium, since it is prevented when calcium chelators, such as EGTA or BAPTA, are present postsynaptically (Llano et al., 1991; Pitler and Alger, 1994). Following induction, DSI can last for up to 2 min. Because the synapses suppressed are inhibitory, DSI moves the postsynaptic cell into a transient state of heightened excitability. Such an alteration in cell excitability could decrease the threshold for action potential firing and may serve to selectively enhance excitatory inputs following depolarization. Decreases in inhibition have been shown to facilitate both NMDA receptor–mediated excitatory currents and the subsequent induction of NMDAR-dependent changes in synaptic strength.

Though the induction of DSI is postsynaptic, its expression appears to be a presynaptic phenomenon as suggested by a lack of change in both quantal size and in postsynaptic cell sensitivity to iontophoresed GABA (Pitler and Alger, 1992; Alger et al., 1996). This implies that, in the production of DSI, information is moving backward across the synapse, from postsynaptic induction to presynaptic expression, and thus that a retrograde message originates in the postsynaptic cell and acts presynaptically to depress presynaptic function (Llano et al., 1991). Hypotheses concerning the identity of the messenger carrying this retrograde signal have centered on glutamate or a glutamate-like substance released from the postsynaptic cell through vesicular fusion and acting through presynaptic metabotropic glutamate receptors (mGluR) (Glitsch et al., 1996; Morishita et al., 1998). In the cerebellum, a selective group II mGluR agonist was shown to occlude DSI expression, and DSI was reduced (although not blocked) in the presence of L-AP3, a group I mGluR antagonist. Curiously, however, the broad-spectrum inhibitor MCPG had no effect on DSI. Similarly, in the hippocampus the mGluR agonist ACPD decreased $GABA_A$ IPSCs and suppressed DSI by ~50%. DSI was proposed to be expressed by glutamate acting on type I mGluRs as the use of group I agonists occluded DSI. Again, however, even very high concentrations of MCPG failed to completely block DSI expression (Morishita et al., 1998).

Now, three new papers have appeared that bring the field to a new high. Two papers appearing in this issue of *Neuron* and one in *Nature* have provided significant insight into the mechanisms of depolarization-induced synapse suppression and have identified the retrograde messenger as being an endogenous cannabinoid (Figure 1). In addition, they contain evidence against a role for glutamate in this retrograde transmission as all three of these studies fail to show an effect of mGluR antagonists on depolarization-induced suppression. Postsynaptically applied botulinum toxin, which blocks vesicle fusion, does not inhibit DSI (Wilson and Nicoll, 2001), making vesicular glutamate release from the postsynaptic cell highly improbable. While synapse suppression is normally induced by postsynaptic depolarization, it is not absolutely required, as postsynaptic liberation of calcium alone, via flash photolysis of caged calcium, was sufficient to induce DSI (Wilson and Nicoll, 2001).

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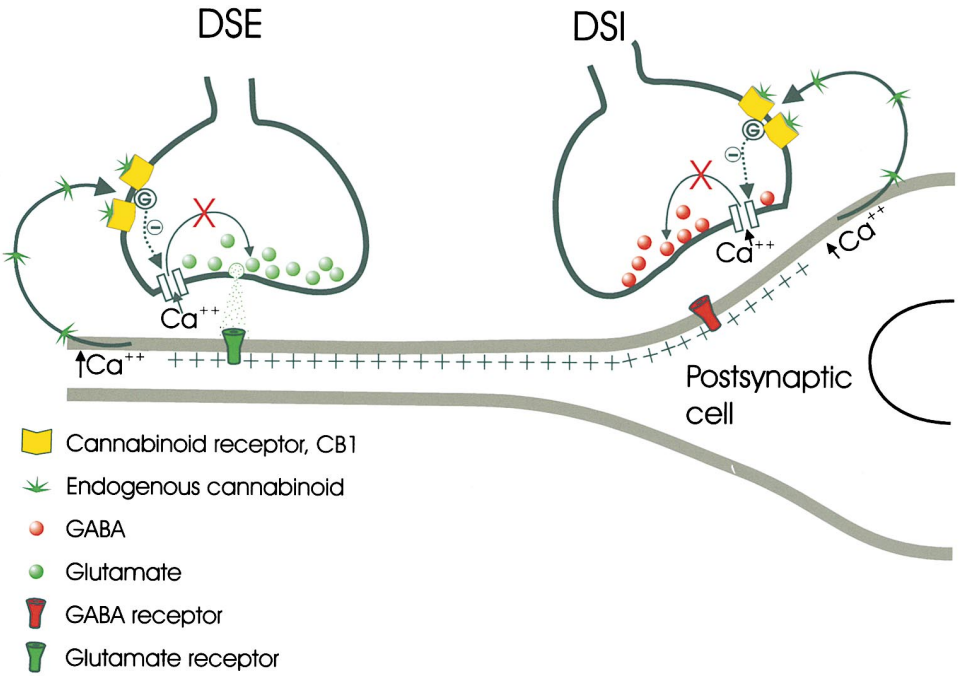

Figure 1. An Endogenous Cannabinoid as the Retrograde Messenger in the Presynaptic Expression of DSI and DSE

A schematic view of the hypothetical mechanism of depolarization-induced suppression of synaptic transmission (DSI/E). Postsynaptic depolarization, provided either by synaptic excitation or by artificial means, leads to a calcium influx in the postsynaptic neuron that stimulates the production of an endogenous cannabinoid. Cannabinoid diffuses from the postsynaptic cell to the presynaptic terminal where it binds to CB1 receptors inducing a G protein–mediated suppression of presynaptic calcium transients, possibly by inhibition of voltage-dependent calcium channels. Suppressed action potential–evoked calcium transients result in a decreased probability of neurotransmitter release. Whether DSI or DSE is expressed in a particular system likely depends primarily on whether the cannabinoid receptors are expressed on excitatory and/or inhibitory presynaptic terminals.

Thus, glutamate release from the postsynaptic cell via a depolarization-induced reversal of a glutamate transporter also seems unlikely. Furthermore, the presynaptic locus of depolarization-induced synaptic suppression is further strengthened in these papers by the finding that paired pulse ratios are altered following postsynaptic depolarization, indicative of a decrease in release probability (Ohno-Shosaku et al., 1998; Kreitzer and Regehr, 2001; Wilson and Nicoll, 2001; but see Morishita and Alger, 1997). DSI in the hippocampus was also able to spread to nearby, undepolarized postsynaptic cells (Wilson and Nicoll, 2001), likely a result of an ability of endogenous cannabinoids to diffuse to nearby synapses.

In retrospect, an endogenous cannabinoid seems an obvious choice as a candidate for the DSI retrograde messenger. Calcium influx often accompanies neuronal depolarization. 2-AG and anandamide are produced in a calcium-dependent fashion, are released following neuronal activity, and may not require special release machinery since they can diffuse from the membrane of the cell (DiMarzo et al., 1998). Cannabinoid receptors are found on the presynaptic terminals of the DSI target cells and are G protein–linked receptors, which likely explains the block of DSI expression by application of G protein inhibitors (Alger and Pitler, 1995). Furthermore, cannabinoids are known to depress transmitter release (Sullivan, 2000). Of course, what is obvious in hindsight was not so obvious before, especially given that there are many potential substances that could fit the bill. The importance of these papers rests, in part, then on the identification of the messenger.

In parallel to the current report in hippocampal slices by Wilson and Nicoll (2001), Ohno-Shosaku et al. (2001) also reveal that the retrograde messenger mediating DSI between cultured dissociated hippocampal neurons is an endogenous cannabinoid. The use of paired recordings by this group showed that a cannabinoid agonist significantly depressed $GABA_A$-mediated synaptic transmission in a presynaptic fashion. DSI could be induced between neuronal pairs independent of whether the postsynaptic cell was excitatory or inhibitory, and was blocked by postsynaptic BAPTA injection. Putting these two experiments together, they then established that an endogenous cannabinoid mediates the depolarization-induced suppression of inhibition. In addition, DSI could be induced between neuronal cell pairs with a more physiological stimulus, that of postsynaptic action potentials. They also report that only 50% of inhibitory neurons have the ability to express DSI. Whether this is also the case between neuronal cell pairs in slices has not been determined. However, that not all interneurons appear to have the ability to express endogenous cannabinoid-mediated DSI is not surprising, given that not all interneurons express the cannabinoid receptor (Katona et al., 1999; Ohno-Shosaku et al., 2001). The physiological consequences of preferential cannabinoid receptor distribution to particular known types of inhibitory in-

terneurons will certainly be of great interest. Such distribution might suggest the specific role that DSI may play in the detailed control of neuronal circuitry.

The paper by Kreitzer and Regehr (2001) is particularly significant because it extends the known phenomenon DSI to excitatory synapses; those formed between climbing or parallel fibers and Purkinje neurons. This suppression of excitatory synapses following postsynaptic depolarization, termed DSE (depolarization-induced suppression of excitation) is also mediated by the retrograde action of an endogenous cannabinoid and appears to be identical in all respects to DSI, save for the fact that the synapse is excitatory (Figure 1). Thus, in DSE, depolarization leads to a decrease in the excitatory synaptic responses mediated by glutamate. Besides extending activity-dependent, cannabinoid-mediated synapse suppression to a new class of synapses, the most significant advance in this paper is that it provides new mechanistic information about this phenomenon. Until now, there were few clues as to how presynaptic function was decreased during DSI expression. Decreased transmitter release could result from a number of factors, including incomplete action potential invasion of the presynaptic terminal, inhibition of presynaptic calcium channels, or direct effects on the release apparatus. Using a combination of imaging and electrophysiological techniques, Kreitzer and Regehr (2001) have demonstrated that cannabinoids act to suppress action potential–evoked calcium rises in the presynaptic terminal, thereby decreasing action potential–evoked transmitter release. Fluorometric imaging of climbing fiber presynaptic terminals using the calcium-sensitive dye fluo-4-dextran revealed that the action potential–evoked rise in intraterminal calcium was decreased by postsynaptic depolarization. This postsynaptic depolarization-induced inhibition of presynaptic calcium was prevented by application of antagonists to the CB1 receptor. Neither incomplete action potential invasion nor branch point failure appeared to cause this decrease in calcium influx since the action potential calcium transients in the terminal were not spatially altered during DSE, only uniformly decreased in intensity. Furthermore, the decreased presynaptic calcium influx was prevented by postsynaptic injection of BAPTA, thereby displaying the postsynaptic calcium-dependence of DSE induction. This extension of cannabinoid-mediated short-term plasticity to an excitatory synapse is a provocative finding and should inspire further investigation as to whether it is a widespread phenomenon throughout the CNS.

How may the retrograde action of endogenous cannabinoid lead to reduced calcium influx and a decrease in transmitter release? Cannabinoid-induced decreases in synaptic transmission have been shown to result from an inhibition of N- and P/Q-type calcium channels (Twitchell et al., 1997; Hoffman and Lupica, 2000), i.e., the subtypes through which calcium influx occurs during evoked transmitter release. Consequently, this leads to a decrease in calcium concentration in the presynaptic terminal and hence a decrease in transmitter release probability. Alger and colleagues have previously reported that blockade of N-type and, under certain conditions, L-type calcium channels abolishes DSI, but inhibition of P- or Q-type channels has no effect (Lenz et al., 1998). Taken together, cannabinoid-induced calcium channel inhibition would certainly result in decreased presynaptic calcium levels, as seen by Kreitzer and Regehr with DSE, and the consequential decrease in release probability. However, questions pertaining to the pathway of DSI expression still remain unanswered, especially in regard to the nature of DSI in the cerebellum. Whether cannabinoids may mediate DSI in the same fashion as in the hippocampus is not known, but given the inability to completely block cerebellar DSI with many inhibitors, including those of mGluRs, arachinidonic acid, nitric oxide, carbon dioxide, adenosine, and GABA (Glitsch et al., 1996), it may seem likely that the retrograde messenger of cerebellar DSI is also an endogenous cannabinoid. However, it should be noted that mechanistic differences between DSI in the cerebellum and the hippocampus have been reported by multiple labs (for example, the mechanism of DSI spread; Alger and Pitler, 1995; Wilson and Nicoll, 2001), and thus results from the hippocampus should only be viewed as clues for the possible mechanisms in the cerebellum.

In summary, increased calcium levels resulting from high levels of postsynaptic activity in both hippocampal pyramidal cells and cerebellar Purkinje cells have been known to lead to an altered state of excitability. The advent of these recent data has revealed endogenous cannabinoids can act in a retrograde fashion to convey information about postsynaptic cell activity to the corresponding presynaptic terminal where DSI/DSE is expressed. Such a novel and exciting finding has significantly raised the understanding of the physiological role of endogenous cannabinoids. Of particular interest from the above studies was the discovery of cerebellar DSE, and this begs the question of whether DSE is also an important phenomenon in other brain areas. At least in the hippocampus, DSE is not found together with DSI (Wagner and Alger, 1996); but in the cerebellum both DSI and DSE are expressed. When found together, can these two forms of synaptic suppression be independently regulated?

Of course, a central question is whether the involvement of endogenous cannabinoids in synapse suppression might help to understand behavioral consequences of marijuana intoxication. Certainly, the prolonged presence in the brain of an exogenous cannabinoid would be expected to activate the DSI/DSE mechanisms, but without the spatial and temporal limits that come with natural activation of the mechanisms. It would not be unexpected that the loss of this spatial/temporal coding could lead to alterations in learning and memory and motor coordination due to marijuana's actions in the hippocampus and cerebellum. Of course, none of this explains the munchies.

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